JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1995, p. 2183–2185 Vol. 33, No. 8
0095-1137/95/$04.00+0

NOTES

Rapid Detection of Methicillin Resistance in Coagulase-Negative Staphylococci by Commercially Available Fluorescence Test

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Received 21 November 1994/Returned for modification 17 January 1995/Accepted 8 May 1995

A commercially available fluorescence test (Crystal MRSA ID System; BBL) for the rapid detection of methicillin resistance in *Staphylococcus aureus* was evaluated for the detection of methicillin resistance in coagulase-negative staphylococci. The assay was compared with the agar dilution method and *mecA* detection by PCR. Provided that one uses an inoculum equivalent to a no. 2 McFarland standard instead of a no. 0.5 McFarland standard, which is used for *S. aureus*, the Crystal MRSA ID System detects methicillin resistance among coagulase-negative staphylococci with sensitivity, specificity, and positive and negative predictive values of 98.5, 95.4, 96.3, and 98.1%, respectively.

Coagulase-negative staphylococci are becoming increasingly important nosocomial pathogens, causing serious infections (4, 10). This is largely due to an increase in the numbers of immunocompromised patients and to the growing use of transient or permanent invasive devices. Moreover, as in *Staphylococcus aureus*, there has been a shift toward more antibiotic-resistant, particularly methicillin-resistant, strains. Treatment of infections caused by such strains requires the use of more toxic and expensive glycopeptides, making the rapid detection of such strains essential.

Until now, standard procedures for the detection of methicillin resistance in clinical microbiology laboratories are susceptibility tests such as disk diffusion, agar dilution, and agar screen methods, but these require 24 h before results are available (7).

Recently, a commercial test (Crystal MRSA ID System; BBL) was introduced for the detection of methicillin resistance in *S. aureus* within 4 h (9). In a recent study this test was also evaluated on a limited number of *S. epidermidis* strains (6).

The purpose of the present study was to evaluate this test for the rapid detection of methicillin resistance among a large number of coagulase-negative staphylococci, including different species, isolated from a variety of clinical specimens.

A total of 240 clinical staphylococcal isolates collected during the periods from October 1991 to March 1992 and from October 1992 to March 1993 at the Laboratory for Microbiology included 119 *S. epidermidis*, 50 *S. haemolyticus*, 35 *S. hominis*, 14 *S. capitis*, 12 *S. warneri*, 5 *S. schleiferi*, and 5 *S. lugdunensis* strains. The organisms were identified by the tube coagulase test and then the rapid identification method developed in our laboratory (3). Only small numbers of *S. capitis*, *S. warneri*, *S. schleiferi*, and *S. lugdunensis* isolates were available, reflecting their low prevalence among coagulase-negative staphylococci. The most common clinical isolates in our hospital are *S. epidermidis* (69.1% of all isolates), *S. haemolyticus* (12.0%), and *S. hominis* (8.8%), a distribution identical to that found by others (2).

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In addition, 30 clinical isolates of *S. aureus* were included; 20 of these isolates were methicillin susceptible (oxacillin MICs, ≤2 μg/ml).

Susceptibility to methicillin was determined by the agar dilution method according to the recommendations of the National Committee for Clinical Laboratory Standards (7) with oxacillin concentrations ranging from 0.0625 to 256 μg/ml. A total of 132 of 240 isolates were identified to be methicillin resistant (oxacillin MICs, ≥4 μg/ml) and 108 of 240 were methicillin susceptible (oxacillin MICs, ≤2 μg/ml). The 132 resistant strains were confirmed to be *mecA* positive by PCR performed as described by Predari et al. (8).

In the commercial qualitative screening test, a fluorescence indicator detects oxygen consumption by actively growing organisms in a microtiter panel. The first well, which is used as a growth control, does not contain any antibiotic, the second well contains oxacillin at a concentration of 4 μg/ml, and the third well, as a negative control, contains vancomycin at a concentration of 16 μg/ml. After incubation the bacteria susceptible to oxacillin do not fluoresce, while resistant organisms, as a result of their metabolic activity, do fluoresce.

Several colonies of the isolates grown on Trypticase soy agar plates were suspended in saline to a density of 0.5 on the McFarland scale, as indicated by the manufacturer, or to a density of 2 on the McFarland scale in the procedure modified by us; 0.5 ml of this suspension was diluted in 3.2 ml of the Crystal MRSA ID broth, and the wells of the panel were inoculated with four drops of the adjusted suspensions.

Fluorescence was detected after 5 h of incubation with a long-wave UV illuminator (365 nm). For the test to be valid the first well should always be positive and the third well should always be negative; the second well is positive if the organism is methicillin resistant and negative if the strain is susceptible.

In a first series of experiments, when the test was applied as described by the manufacturer, e.g., starting with an inoculum corresponding to 0.5 on the McFarland scale, all but two resistant strains among the 121 non-*S. epidermidis* isolates were correctly identified by the new method after 5 h of incubation, representing a 96.6% sensitivity and a 100% specificity. Upon repeat testing, the two initially false methicillin-susceptible results (1 *S. hominis* and 1 *S. haemolyticus*) were resolved.

TABLE 1. Detection of *mecA* gene by PCR and methicillin susceptibility testing of coagulase-negative staphylococci by the Crystal MRSA ID System

Species (no. of isolates tested)	No. of isolates with the indicated characteristics tested at the following inoculum:							
	0.5 McFarland standard				2.0 McFarland standard			
	mec gene positive[a]		*mec* gene negative[b]		*mec* gene positive		*mec* gene negative	
	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
S. haemolyticus (50)	1	42	7	0	0	43	7	0
S. hominis (35)	1	15	19	0	0	16	18	1
S. capitis (14)	0	0	14	0	0	0	14	0
S. warneri (12)	0	0	12	0	0	0	11	1
S. lugdunensis (5)	0	0	5	0	0	0	5	0
S. schleiferi (5)	0	0	5	0	0	0	4	1
S. epidermidis (119)	26	47	46	0	2	71	44	2

[a] For all *mecA* gene-positive strains, oxacillin MICs were $\geq$4 μg/ml.
[b] For all *mecA* gene-negative strains, oxacillin MICs were $\leq$2 μg/ml.

The *S. epidermidis* isolates included 46 methicillin-susceptible strains, all of which were correctly identified by the BBL Crystal MRSA ID System. However, only 47 of the 73 methicillin-resistant *S. epidermidis* strains were correctly identified, resulting in a sensitivity of 64.4%.

Upon repeat testing only 4 of the 26 false-negative results were resolved, resulting in a sensitivity of 69.9%. We hypothesized that this lack of sensitivity was due to the slower growth of many *S. epidermidis* isolates. An increase in the incubation time to 8 and 20 h did not overcome the problem.

Therefore, in a second series of experiments, the effect of the heavier inoculum on the results of the Crystal ID MRSA System was evaluated with all of the coagulase-negative staphylococcal isolates as well as with 30 *S. aureus* isolates.

When all *mecA* gene-negative coagulase-negative staphylococcal isolates were negative in the Crystal MRSA ID System with an inoculum corresponding to 0.5 on the McFarland scale (Table 1), resulting in a specificity of 100%, an increase in the inoculum density to 2 on the McFarland scale produced a total of 5 false-positive results for methicillin resistance among the 240 strains tested, resulting in a specificity of 95.4%; 3 of these false-positive results for methicillin resistance were detected among the 121 non-*S. epidermidis* strains (1 *S. hominis*, 1 *S. warneri*, and 1 *S. schleiferi*) and 2 were detected among the 119 *S. epidermidis* strains.

All *mecA* gene-positive, non-*S. epidermidis* coagulase-negative staphylococci (43 *S. haemolyticus* strains and 16 *S. hominis* strains) were also positive in the Crystal MRSA ID System with a no. 2 McFarland inoculum, resulting in a sensitivity of 100% compared with the initial sensitivity of 96.6% when the test was applied with the lower inoculum.

For *S. epidermidis*, 71 of 73 of the *mecA* gene-positive isolates were detected when the higher inoculum was used, increasing the initial sensitivity of the test from 64 to 97.2%.

Thus, overall there was a close correlation between the presence of the *mecA* gene and a positive reaction in the modified Crystal MRSA ID System when it was used to test coagulase-negative staphylococci. However, when this higher inoculum was tested with the 30 *S. aureus* isolates, it produced an unacceptable rate of 65% false-positive methicillin resistance. In practice, for the presumptive detection of methicillin-resistant staphylococci by the Crystal MRSA ID System, the distinction between *S. aureus* and coagulase-negative staphylococci should first be made either by a slide coagulase test or a tube coagulase test, and the inoculum should be adapted as a function of the result of that test.

All of the conventional methods such as the disk diffusion method, MIC determinations, and methicillin and oxacillin agar plate screenings are growth dependent and therefore require 24 h of incubation. Besides, 16 of the 26 initially undetected methicillin-resistant *S. epidermidis* isolates were also misidentified as methicillin susceptible in the disk diffusion test (data not shown). Other studies also documented problems with the detection of methicillin resistance among coagulase-negative staphylococci by the disk diffusion test (1, 2, 5). Therefore, alternative or supplementary methods are desirable for the confirmation of methicillin resistance in staphylococci. We hypothesized that the Crystal MRSA ID System might serve as such an alternative or as a supplementary method for the detection of methicillin resistance in coagulase-negative staphylococci, provided that a modification of the test is introduced. Recently, this test has been evaluated for the detection of methicillin resistance in *S. aureus* (4, 7) and in a limited number of *S. epidermidis* strains (4). In the present study this test was evaluated with a large number of coagulase-negative staphylococci, including the most clinically relevant species.

In conclusion, when used in combination with a coagulase slide or tube test, the Crystal MRSA ID System can be performed with an inoculum of either 0.5 or 2.0 on the McFarland scale for *S. aureus* or coagulase-negative staphylococci, respectively, to identify methicillin resistance among coagulase-negative staphylococci with an overall sensitivity of >95% 18 to 20 h earlier than the conventional methods.

The test is simple to perform in any laboratory, thus providing the clinician earlier information and allowing better patient management.

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